Serial No.: 10/066,432 Filed: January 31, 2002

Page : 4 of 26

In the Claims:

Please cancel claims 1-53. Please add new claims 54-136. The claims and their status are shown below.

1-53. (Canceled)

, 54. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said pair of HSV DNA polymerase primers comprises a first HSV DNA polymerase primer and a second HSV DNA polymerase primer, wherein said first HSV DNA polymerase primer comprises the sequence 5'-GCT CGA GTG CGA AAA AAC GTT C-3' (SEQ ID NO:1), wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize to said amplification product within no more than five nucleotides of each other, wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of fluorescence resonance energy transfer (FRET) between said donor fluorescent moiety of said first HSV DNA polymerase probe and said acceptor fluorescent moiety of said second HSV DNA polymerase probe,

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

- 55. (New) The method of claim 54, wherein said second HSV DNA polymerase primer comprises the sequence 5'-CGG GGC GCT CGG CTA AC-3' (SEQ ID NO:2).
- /56. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

Serial No.: 10/066,432 : January 31, 2002 Filed

Page : 5 of 26

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said pair of HSV DNA polymerase primers comprises a first HSV DNA polymerase primer and a second HSV DNA polymerase primer, wherein said second HSV DNA polymerase primer comprises the sequence 5'-CGG GGC GCT CGG CTA AC-3' (SEO ID NO:2), wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize to said amplification product within no more than five nucleotides of each other, wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a corresponding acceptor fluorescent moiety; and detecting the presence or absence of FRET between said donor fluorescent moiety of said first HSV DNA polymerase probe and said acceptor fluorescent moiety of said second

HSV DNA polymerase probe,

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

- 57. (New) The method of claim 56, wherein said first HSV DNA polymerase primer comprises the sequence 5'-GCT CGA GTG CGA AAA AAC GTT C-3' (SEQ ID NO:1).
- 58. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize to said amplification product within no more than five nucleotides of each other,

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 6 of 26

wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a corresponding acceptor fluorescent moiety, wherein said first HSV DNA polymerase probe comprises the sequence 5'-GCG CAC CAG ATC CAC GCC CTT GAT GAG C-3' (SEQ ID NO:3); and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first HSV DNA polymerase probe and said acceptor fluorescent moiety of said second HSV DNA polymerase probe,

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

- 59. (New) The method of claim 58, wherein said second HSV DNA polymerase probe comprises the sequence 5'-CTT GCC CCC GCA GAT GAC GCC- 3' (SEQ ID NO:4).
- 60. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize to said amplification product within no more than five nucleotides of each other, wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probe of said pair of HSV DNA polymerase probe comprises the sequence 5'-CTT GCC CCC GCA GAT GAC GCC-3' (SEQ ID NO:4); and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first HSV DNA polymerase probe and said acceptor fluorescent moiety of said second HSV DNA polymerase probe,

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 7 of 26

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

- 61. (New) The method of claim 60, wherein said first HSV DNA polymerase probe comprises the sequence 5'-GCG CAC CAG ATC CAC GCC CTT GAT GAG C- 3' (SEQ ID NO:3).
- 62. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize to said amplification product within no more than five nucleotides of each other, wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a corresponding acceptor fluorescent moiety, wherein said first HSV DNA polymerase probe comprises the sequence 5'-GTA CAT CGG CGT CAT CTG CGG GGG CAA G-3' (SEQ ID NO:5); and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first HSV DNA polymerase probe and said acceptor fluorescent moiety of said second HSV DNA polymerase probe,

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

63. (New) The method of claim 62, wherein said second HSV DNA polymerase probe comprises the sequence 5'- T GCT CAT CAA GGG CGT GGA TCT GGT GC- 3' (SEQ ID NO:6).

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 8 of 26

/ 64. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize to said amplification product within no more than five nucleotides of each other, wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a corresponding acceptor fluorescent moiety, wherein said second HSV DNA polymerase probe comprises the sequence 5'- T GCT CAT CAA GGG CGT GGA TCT GGT GC-3' (SEQ ID NO:6); and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first HSV DNA polymerase probe and said acceptor fluorescent moiety of said second HSV DNA polymerase probe,

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

- 65. (New) The method of claim 64, wherein said first HSV DNA polymerase probe comprises the sequence 5'-GTA CAT CGG CGT CAT CTG CGG GGG CAA G- 3' (SEQ ID NO:5).
- 66. (New) The method of claim 54, 56, 58, 60, 62, or 64, wherein the presence of said FRET in an amount at least 3 times the amount of FRET in a sample lacking said HSV HSV DNA polymerase nucleic acid molecule is indicative of the presence of an HSV infection in said individual.
- 67. (New) The method of claim 54, 56, 58, 60, 62, or 64, wherein said cycling step is performed on a control sample.

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 9 of 26

68. (New) The method of claim 67, wherein said control sample comprises a portion of said HSV DNA polymerase nucleic acid molecule.

- 69. (New) The method of claim 54, 56, 58, 60, 62, or 64, wherein said cycling step uses a pair of control primers and a pair of control probes, wherein said control primers and said control probes are other than said HSV DNA polymerase primers and said HSV DNA polymerase probes, wherein a control amplification product is produced if control template is present in said sample, wherein said control probes hybridize to said control amplification product.
 - 70. (New) The method of claim 54, 56, 58, 60, 62, or 64, further comprising:

performing at least one cycling step, wherein said cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if an HSV TK nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of TK probes, wherein the members of said pair of TK probes hybridize within no more than five nucleotides of each other, wherein a first TK probe of said pair of TK probes is labeled with a donor fluorescent moiety and wherein a second TK probe of said pair of TK probes is labeled with a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first TK probe and said acceptor fluorescent moiety of said second TK probe upon hybridization of said pair of TK probes to said targets.

- 71. (New) The method of claim 70, wherein said pair of TK primers comprises a first TK primer and a second TK primer, wherein said first TK primer comprises the sequence 5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G, and wherein said second TK primer comprises the sequence 5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C.
- 72. (New) The method of claim 70, wherein said first TK probe comprises the sequence 5'-CGC GCG ACG ATA TCG TCT ACG TAC- 3' (SEQ ID NO:9), and wherein said second TK probe comprises the sequence 5'-CGA GCC GAT GAC TTA CTG GCA GGT G- 3' (SEQ ID NO:10).

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 10 of 26

73. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if a TK HSV nucleic acid molecule is present in said sample, wherein said pair of TK primers comprises a first TK primer and a second TK primer, wherein said first TK primer comprises the sequence 5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G, wherein said hybridizing step comprises contacting said sample with a pair of TK probes, wherein the members of said pair of TK probes hybridize within no more than five nucleotides of each other, wherein a first TK probe of said pair of TK probes is labeled with a donor fluorescent moiety and wherein a second TK probe of said pair of TK probes is labeled with a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first TK probe and said acceptor fluorescent moiety of said second TK probe upon hybridization of said pair of TK probes to said targets,

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

- 74. (New) The method of claim 73, wherein said second TK primer comprises the sequence 5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C.
- 75. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if a TK HSV nucleic acid molecule is present in said sample, wherein said pair of TK primers comprises a first TK primer and a second TK primer, wherein said second TK primer comprises the sequence 5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C, wherein said

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 11 of 26

hybridizing step comprises contacting said sample with a pair of TK probes, wherein the members of said pair of TK probes hybridize within no more than five nucleotides of each other, wherein a first TK probe of said pair of TK probes is labeled with a donor fluorescent moiety and wherein a second TK probe of said pair of TK probes is labeled with a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first TK probe and said acceptor fluorescent moiety of said second TK probe upon hybridization of said pair of TK probes to said targets,

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

- 76. (New) The method of claim 75, wherein said first TK primer comprises the sequence 5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G.
- 77. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if a TK HSV nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of TK probes, wherein the members of said pair of TK probes hybridize within no more than five nucleotides of each other, wherein a first TK probe of said pair of TK probes is labeled with a donor fluorescent moiety and wherein a second TK probe of said pair of TK probes is labeled with a corresponding acceptor fluorescent moiety, wherein said first TK probe comprises the sequence 5'-CGC GCG ACG ATA TCG TCT ACG TAC- 3' (SEQ ID NO:9); and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first TK probe and said acceptor fluorescent moiety of said second TK probe upon hybridization of said pair of TK probes to said targets,

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 12 of 26

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

78. (New) The method of claim 77, wherein said second TK probe comprises the sequence 5'-CGA GCC GAT GAC TTA CTG GCA GGT G- 3' (SEQ ID NO:10).

79. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if a TK HSV nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a pair of TK probes, wherein the members of said pair of TK probes hybridize within no more than five nucleotides of each other, wherein a first TK probe of said pair of TK probes is labeled with a donor fluorescent moiety and wherein a second TK probe of said pair of TK probes is labeled with a corresponding acceptor fluorescent moiety, wherein said second TK probe comprises the sequence 5'-CGA GCC GAT GAC TTA CTG GCA GGT G- 3' (SEQ ID NO:10); and

detecting the presence or absence of FRET between said donor fluorescent moiety of said first TK probe and said acceptor fluorescent moiety of said second TK probe upon hybridization of said pair of TK probes to said targets,

wherein the presence of FRET is indicative of the presence of HSV in said biological sample, and wherein the absence of FRET is indicative of the absence of HSV in said biological sample.

- 80. (New) The method of claim 79, wherein said first TK probe comprises the sequence 5'-CGC GCG ACG ATA TCG TCT ACG TAC- 3' (SEQ ID NO:9).
- 81. (New) The method of claim 73, 75, 77, or 79, wherein the presence of said FRET in an amount at least 3 times the amount of FRET in a sample lacking said HSV TK nucleic acid molecule is indicative of the presence of an HSV infection in said individual.
- 82. (New) The method of claim 73, 75, 77, or 79, wherein said cycling step is performed on a control sample.

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 13 of 26

83. (New) The method of claim 82, wherein said control sample comprises a portion of said TK HSV nucleic acid molecule.

- 84. (New) The method of claim 73, 75, 77, or 79, wherein said cycling step uses a pair of control primers and a pair of control probes, wherein said control primers and said control probes are other than said TK primers and said TK probes, wherein a control amplification product is produced if control template is present in said sample, wherein said control probes hybridize to said control amplification product.
- 85. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the members of said pair of probes hybridize within no more than two nucleotides of each other.
- 86. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the members of said pair of probes hybridize within no more than one nucleotide of each other.
- 87. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein said donor fluorescent moiety is fluorescein.
- 88. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein said detecting step comprises exciting said biological sample at a wavelength absorbed by said donor fluorescent moiety and visualizing and/or measuring the wavelength emitted by said acceptor fluorescent moiety.
- 89. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein said detecting comprises quantitating said FRET.
- 90. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein said detecting step is performed after each cycling step.
- 91. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein said detecting step is performed in real time.
- 92. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the amplification product differs in sequence between HSV-1 and HSV-2 by at least one nucleotide.
- 93. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, further comprising determining the melting temperature between one or both of said probe(s) and said amplification product, wherein said melting temperature confirms said presence or said absence of said HSV.

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 14 of 26

94. (New) The method of claim 93, wherein said melting temperature distinguishes between HSV-1 and HSV-2.

- 95. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, further comprising preventing amplification of a contaminant nucleic acid.
- 96. (New) The method of claim 95, wherein said preventing comprises performing said amplifying step in the presence of uracil.
- 97. (New) The method of claim 96, wherein said preventing further comprises treating said biological sample with uracil-DNA glycosylase prior to a first amplification step.
- 98. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein said biological sample is selected from the group consisting of an ocular swab, a genital specimen, a dermal specimen, a pap smear, amniotic fluid and cerebrospinal fluid.
- 99. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the presence of said FRET within 10 cycles is indicative of the presence of an HSV infection in said individual.
- 100. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the presence of said FRET within 20 cycles is indicative of the presence of an HSV infection in said individual.
- 101. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the presence of said FRET within 30 cycles is indicative of the presence of an HSV infection in said individual.
- 102. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the presence of said FRET within 37 cycles is indicative of the presence of an HSV infection in said individual.
- 103. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the absence of said FRET within 37 cycles is indicative of the absence of an HSV infection in said individual.
- 104. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the presence of said FRET within 40 cycles is indicative of the presence of an HSV infection in said individual.

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 15 of 26

105. (New) The method of claim 54, 56, 58, 60, 62, 64, 73, 75, 77, or 79, wherein the presence of said FRET within 50 cycles is indicative of the presence of an HSV infection in said individual.

/ 106. (New) A method of distinguishing between HSV-1 and HSV-2 in a biological sample from an individual, the method comprising:

performing at least one cycling step, wherein said cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV-1 DNA polymerase amplification product if an HSV-1 DNA polymerase nucleic acid molecule is present in said sample and/or an HSV-2 DNA polymerase amplification product if an HSV-2 DNA polymerase nucleic acid molecule is present in said sample, wherein said HSV-1 and said HSV-2 amplification products differ in sequence by at least one nucleotide, wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize within no more than five nucleotides of each other, wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a corresponding acceptor fluorescent moiety, wherein said first HSV DNA polymerase probe comprises a sequence selected from the group consisting of 5'-GCG CAC CAG ATC CAC GCC CTT GAT GAG C-3' (SEQ ID NO:3) and 5'-GTA CAT CGG CGT CAT CTG CGG GGG CAA G-3' (SEQ ID NO:5);

detecting the presence or absence of FRET between said donor fluorescent moiety of said first HSV DNA polymerase probe and said corresponding acceptor fluorescent moiety of said second HSV DNA polymerase probe, wherein the presence of FRET is indicative of the presence of HSV in said biological sample, wherein the absence of FRET is indicative of the absence of HSV in said biological sample; and

determining the melting temperature between one or both of said HSV DNA polymerase probes and said HSV DNA polymerase amplification products, wherein said melting temperature distinguishes between HSV-1 and HSV-2.

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 16 of 26

107. (New) The method of claim 106, wherein said second HSV DNA polymerase probe comprises a sequence selected from the group consisting of 5'-CTT GCC CCC GCA GAT GAC GCC-3' (SEQ ID NO:4) and 5'-T GCT CAT CAA GGG CGT GGA TCT GGT GC-3' (SEQ ID NO:6).

√ 108. (New) A method of distinguishing between HSV-1 and HSV-2 in a biological sample from an individual, the method comprising:

performing at least one cycling step, wherein said cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV-1 DNA polymerase amplification product if an HSV-1 DNA polymerase nucleic acid molecule is present in said sample and/or an HSV-2 DNA polymerase amplification product if an HSV-2 DNA polymerase nucleic acid molecule is present in said sample, wherein said HSV-1 and said HSV-2 amplification products differ in sequence by at least one nucleotide, wherein said hybridizing step comprises contacting said sample with a pair of HSV DNA polymerase probes, wherein the members of said pair of HSV DNA polymerase probes hybridize within no more than five nucleotides of each other, wherein a first HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a donor fluorescent moiety and wherein a second HSV DNA polymerase probe of said pair of HSV DNA polymerase probes is labeled with a corresponding acceptor fluorescent moiety, wherein said second HSV DNA polymerase probe comprises a sequence selected from the group consisting of 5'-CTT GCC CCC GCA GAT GAC GCC-3' (SEQ ID NO:4) and 5'- T GCT CAT CAA GGG CGT GGA TCT GGT GC-3' (SEQ ID NO:6);

detecting the presence or absence of FRET between said donor fluorescent moiety of said first HSV DNA polymerase probe and said corresponding acceptor fluorescent moiety of said second HSV DNA polymerase probe, wherein the presence of FRET is indicative of the presence of HSV in said biological sample, wherein the absence of FRET is indicative of the absence of HSV in said biological sample; and

determining the melting temperature between one or both of said HSV DNA polymerase probes and said HSV DNA polymerase amplification products, wherein said melting temperature distinguishes between HSV-1 and HSV-2.

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 17 of 26

109. (New) The method of claim 108, wherein said first HSV DNA polymerase probe comprises a sequence selected from the group consisting of 5'-GCG CAC CAG ATC CAC GCC CTT GAT GAG C-3' (SEQ ID NO:3) and 5'-GTA CAT CGG CGT CAT CTG CGG GGG CAA G-3' (SEQ ID NO:5).

110. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said pair of HSV DNA polymerase primers comprises a first HSV DNA polymerase primer and a second HSV DNA polymerase primer, wherein said first HSV DNA polymerase primer comprises the sequence 5'-GCT CGA GTG CGA AAA AAC GTT C-3' (SEQ ID NO:1), wherein said hybridizing step comprises contacting said sample with an HSV DNA polymerase probe, wherein said HSV DNA polymerase probe is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety and said acceptor fluorescent moiety of said HSV DNA polymerase probe,

wherein the presence or absence of FRET is indicative of the presence or absence of HSV in said sample.

- 111. (New) The method of claim 110, wherein said second HSV DNA polymerase primer comprises the sequence 5'-CGG GGC GCT CGG CTA AC-3' (SEQ ID NO:2).
- /112. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said pair of HSV DNA polymerase primers comprises a first HSV DNA polymerase primer and a second HSV DNA polymerase primer, wherein said second HSV DNA

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 18 of 26

polymerase primer comprises the sequence 5'-CGG GGC GCT CGG CTA AC-3' (SEQ ID NO:2), wherein said hybridizing step comprises contacting said sample with an HSV DNA polymerase probe, wherein said HSV DNA polymerase probe is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety and said acceptor fluorescent moiety of said HSV DNA polymerase probe,

wherein the presence or absence of FRET is indicative of the presence or absence of HSV in said sample.

- 113. (New) The method of claim 112, wherein said first HSV DNA polymerase primer comprises the sequence 5'-GCT CGA GTG CGA AAA AAC GTT C-3' (SEQ ID NO:1).
- 114. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with an HSV DNA polymerase probe, wherein said HSV DNA polymerase probe is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety, wherein said HSV DNA polymerase probe comprises a sequence selected from the group consisting of 5'-GCG CAC CAG ATC CAC GCC CTT GAT GAG C-3' (SEQ ID NO:3), 5'-CTT GCC CCC GCA GAT GAC GCC-3' (SEQ ID NO:4), 5'-GTA CAT CGG CGT CAT CTG CGG GGG CAA G-3' (SEQ ID NO:5), and 5'-T GCT CAT CAA GGG CGT GGA TCT GGT GC-3' (SEQ ID NO:6); and

detecting the presence or absence of FRET between said donor fluorescent moiety and said acceptor fluorescent moiety of said HSV DNA polymerase probe,

wherein the presence or absence of FRET is indicative of the presence or absence of HSV in said sample.

115. (New) The method of claim 114, wherein said first and second HSV DNA polymerase primers comprise the sequences 5'-GCT CGA GTG CGA AAA AAC GTT C-3' (SEQ ID NO:1) and 5'-CGG GGC GCT CGG CTA AC-3' (SEQ ID NO:2), respectively.

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 19 of 26

116. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if an HSV TK nucleic acid molecule is present in said sample, wherein said pair of TK primers comprises a first TK primer and a second TK primer, wherein said first TK primer comprises the sequence 5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G, wherein said hybridizing step comprises contacting said sample with a TK probe, wherein said TK probe is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety and said acceptor fluorescent moiety of said TK probe,

wherein the presence or absence of FRET is indicative of the presence or absence of HSV in said sample.

- 117. (New) The method of claim 116, wherein said second TK primer comprises the sequence 5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C.
- 118. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if an HSV TK nucleic acid molecule is present in said sample, wherein said pair of TK primers comprises a first TK primer and a second TK primer, wherein said second TK primer comprises the sequence 5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C, wherein said hybridizing step comprises contacting said sample with a TK probe, wherein said TK probe is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety; and

detecting the presence or absence of FRET between said donor fluorescent moiety and said acceptor fluorescent moiety of said TK probe,

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 20 of 26

wherein the presence or absence of FRET is indicative of the presence or absence of HSV in said sample.

119. (New) The method of claim 118, wherein said first TK primer comprises the sequence 5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G.

120. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a hybridizing step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if an HSV TK nucleic acid molecule is present in said sample, wherein said hybridizing step comprises contacting said sample with a TK probe, wherein said TK probe is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety, wherein said TK probe comprises a sequence selected from the group consisting of 5'-CGC GCG ACG ATA TCG TCT ACG TAC- 3' (SEQ ID NO:9) and 5'-CGA GCC GAT GAC TTA CTG GCA GGT G- 3' (SEQ ID NO:10); and

detecting the presence or absence of FRET between said donor fluorescent moiety and said acceptor fluorescent moiety of said TK probe,

wherein the presence or absence of FRET is indicative of the presence or absence of HSV in said sample.

- 121. (New) The method of claim 120, wherein said first and second TK primers comprise the sequences 5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G, and 5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C.
- 122. (New) The method of claim 110, 112, 114, 116, 118, or 120, wherein said amplification employs a polymerase enzyme having 5' to 3' exonuclease activity.
- 123. (New) The method of claim 110, 112, 114, 116, 118, or 120, wherein said donor and acceptor fluorescent moieties are within no more than 5 nucleotides of each other on said probe.
- 124. (New) The method of claim 123, wherein said acceptor fluorescent moiety is a quencher.

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 21 of 26

125. (New) The method of claim 110, 112, 114, 116, 118, or 120, wherein said probe comprises a nucleic acid sequence that permits secondary structure formation, wherein said secondary structure formation results in spatial proximity between said donor and said acceptor fluorescent moiety.

- 126. (New) The method of claim 125, wherein said acceptor fluorescent moiety is a quencher.
- 127. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a dye-binding step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said pair of HSV DNA polymerase primers comprises a first HSV DNA polymerase primer and a second HSV DNA polymerase primer, wherein said first HSV DNA polymerase primer comprises the sequence 5'-GCT CGA GTG CGA AAA AAC GTT C-3' (SEQ ID NO:1), wherein said dye-binding step comprises contacting said HSV DNA polymerase amplification product with a nucleic acid binding dye; and

detecting the presence or absence of binding of said nucleic acid binding dye to said amplification product,

wherein the presence of binding is indicative of the presence of HSV in said sample, and wherein the absence of binding is indicative of the absence of HSV in said sample.

- 128. (New) The method of claim 127, wherein said second HSV DNA polymerase primer comprises the sequence 5'-CGG GGC GCT CGG CTA AC-3' (SEQ ID NO:2).
- 129. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a dye-binding step, wherein said amplifying step comprises contacting said sample with a pair of HSV DNA polymerase primers to produce an HSV DNA polymerase amplification product if an HSV HSV DNA polymerase nucleic acid molecule is present in said sample, wherein said pair of HSV DNA polymerase primers comprises a first HSV DNA

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 22 of 26

polymerase primer and a second HSV DNA polymerase primer, wherein said second HSV DNA polymerase primer comprises the sequence 5'-CGG GGC GCT CGG CTA AC-3' (SEQ ID NO:2), wherein said dye-binding step comprises contacting said HSV DNA polymerase amplification product with a nucleic acid binding dye; and

detecting the presence or absence of binding of said nucleic acid binding dye to said amplification product,

wherein the presence of binding is indicative of the presence of HSV in said sample, and wherein the absence of binding is indicative of the absence of HSV in said sample.

- 130. (New) The method of claim 129, wherein said first HSV DNA polymerase primer comprises the sequence 5'-GCT CGA GTG CGA AAA AAC GTT C-3' (SEQ ID NO:1).
- / 131. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a dye-binding step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if an HSV TK nucleic acid molecule is present in said sample, wherein said pair of TK primers comprises a first TK primer and a second TK primer, wherein said first TK primer comprises the sequence 5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G, wherein said dye-binding step comprises contacting said TK amplification product with a nucleic acid binding dye; and

detecting the presence or absence of binding of said nucleic acid binding dye to said amplification product,

wherein the presence of binding is indicative of the presence of HSV in said sample, and wherein the absence of binding is indicative of the absence of HSV in said sample.

- 132. (New) The method of claim 131, wherein said second TK primer comprises the sequence 5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C.
- 133. (New) A method for detecting the presence or absence of HSV in a biological sample from an individual, said method comprising:

Serial No.: 10/066,432 Filed: January 31, 2002

Page : 23 of 26

performing at least one cycling step, wherein a cycling step comprises an amplifying step and a dye-binding step, wherein said amplifying step comprises contacting said sample with a pair of TK primers to produce a TK amplification product if an HSV TK nucleic acid molecule is present in said sample, wherein said pair of TK primers comprises a first TK primer and a second TK primer, wherein said second TK primer comprises the sequence 5'-TTG TTA TCT GGG CGC TMG TCA TT-3' (SEQ ID NO:8), wherein M is A or C, wherein said dye-binding step comprises contacting said TK amplification product with a nucleic acid binding dye; and

detecting the presence or absence of binding of said nucleic acid binding dye to said amplification product,

wherein the presence of binding is indicative of the presence of HSV in said sample, and wherein the absence of binding is indicative of the absence of HSV in said sample.

- 134. (New) The method of claim 133, wherein said first TK primer comprises the sequence 5'-CAC GCT RCT GCG GGT TTA TAT AGA-3' (SEQ ID NO:7), wherein R is A or G.
- 135. (New) The method of claim 127, 129, 131, 133, wherein said nucleic acid binding dye is ethidium bromide.
- 136. (New) The method of claim 127, 129, 131, 133, further comprising determining the melting temperature between said amplification product and said nucleic acid binding dye, wherein said melting temperature confirms said presence or absence of said HSV.